Categories and Inheritance of Resistance to Russian Wheat Aphid (Homoptera: Aphididae) Biotype 2 in a Selection from Wheat Cereal Introduction 2401

PRIYAMVADA VOOTHULURU,[1] JIANYE MENG,[2] CHITVAN KHAJURIA,[3] JOE LOUIS,[3] LIECENG ZHU,[3] SHARON STARKEY,[3] GERALD E. WILDE,[3] CHERYL A. BAKER,[4] AND C. MICHAEL SMITH[3,5]

J. Econ. Entomol. 99(5): 1854–1861 (2006)

ABSTRACT The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), is one of the most devastating insect pests of wheat (*Triticum* spp.) and barley (*Hordeum* spp.) in the world. Yield losses and control costs are valued at several hundred million dollars each year. The use of *D. noxia*-resistant cultivars is an ecologically, economically, and biologically sound method of managing this pest. Several *D. noxia* resistance (*Dn*) genes from wheat have been used to develop cultivars resistant to *D. noxia*. However, a new U.S. *D. noxia* biotype (biotype 2) in Colorado is virulent to all known *Dn* genes except the *Dn7* gene from rye (*Secale* spp.). Hence, there is an immediate need to identify and characterize unique sources of *D. noxia* resistance to biotypes. In this article, we report resistance to *D. noxia* biotype 2, identified in a selection from wheat cereal introduction (CItr) 2401, that is controlled by two dominant genes. CItr2401 has a strong antibiosis effect that is exhibited as a reduced intrinsic rate of increase of *D. noxia* biotype 2. CItr2401 plants also exhibit tolerance to leaf rolling and chlorosis. No antixenosis was detected in CItr2401.

KEY WORDS *Diuraphis noxia*, insect resistance categories, antibiosis, proportional plant dry weight loss, tolerance index

Wheat (*Triticum* spp.) is one of the most important food crops and is a dietary staple of more than one-half of the world's population (Johnson et al. 1978). The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), is a devastating pest of both wheat and barley (*Hordeum* spp.). *D. noxia* is indigenous to Afghanistan, Iran, southern Russia, and countries bordering the Mediterranean Sea (Hewitt et al. 1984), and it was first reported in North America in 1986 (Stoetzel 1987). The cumulative losses to all U.S. small grain production due to *D. noxia* control, grain losses, and lost community economic activity from 1986 to 1993 was valued at ≈$1 billion (Morrison and Peairs 1998).

The characteristic symptoms of *D. noxia* damage are leaf rolling, leaf folding, interveinal chlorosis, and, in severe infestations, plant death (Walters et al. 1980). The aphids inject salivary enzymes into plant leaves and suck the phloem sap from leaves while feeding. These enzymes break down leaf chloroplasts, resulting in white, yellow, or purple longitudinal leaf streaks (Fouche et al. 1984, Pike et al. 1991), and ultimately, reduced photosynthetic efficiency of the plant, reduced vigor, and increased sensitivity to environmental stresses. Under heavy infestation, grain weights can be reduced by as much as 80% of normal yield (Hein 1992). Both temperature and host plant age influence the rate of *D. noxia* development, fecundity, and longevity. A temperature regime of 18–21°C and a growth stage of 30 (Zadoks et al. 1974) are the optimal conditions for a high intrinsic rate of *D. noxia* increase (Girma et al. 1990).

Systemic insecticides have provided acceptable *D. noxia* control (Webster et al. 1987), and in some systems, cultural practices and biological control have been used to manage *D. noxia* (Prinsloo 2000, Wraight et al. 1993). However, the habit of *D. noxia* seeking refuge in rolled leaves of damaged plants often complicates the effective management of *D. noxia* with insecticides or biocontrol agents. Hence, the use of *D. noxia*-resistant cultivars is an ecologically, economically, and biologically sound method of managing this pest.

One of the prerequisites for developing arthropod-resistant cultivars is the identification and categorization of sources of resistance. More than 30,000 accessions of wheat and related cereal crops have been

[1] Division of Plant Sciences, University of Missouri, Columbia, MO 65201.
[2] Department of Agronomy, Kansas State University, Manhattan, KS 66506.
[3] Department of Entomology, Kansas State University, Manhattan, KS 66506-4004.
[4] USDA–ARS, Plant Science Research Laboratory, 1301 N. Western, Stillwater, OK 74075.
[5] Corresponding author, e-mail: cmsmith@ksu.edu.

evaluated for *D. noxia* resistance worldwide, and ≈100 resistant sources have been identified that have considerable *D. noxia* resistance (Souza 1998, Webster and Kenkel 1999). Ten *Dn* genes from wheat and one gene from rye controlling resistance to *D. noxia* have been characterized. The mode of inheritance as well as the chromosome location of these genes in the wheat genome has been determined (Liu et al. 2002, 2005). The *D. noxia*-resistant 'Halt', 'Prairie Red', 'Yumar', and 'Prowers 99' were developed using wheat PI372129, and 'Stanton' was developed using resistance in wheat PI220350 (Quick et al. 1996, Martin et al. 2001). Souza et al. (2002) reported the development of wheat germplasm with *D. noxia* resistance derived from backcrosses of the hard red winter wheat 'Manning' with PI47545, PI48650, and PI94460. Resistance to *D. noxia* in Iran has been identified in both tetraploid and hexaploid wheat genotypes (Assad 2002, Estakhr and Assad 2002).

Unfortunately, a new North American *D. noxia* biotype was reported and designated as biotype 2 in southeastern Colorado in 2003 (Haley et al. 2004). Biotype 2 is virulent to all known *Dn* genes except the *Dn7* gene originating from rye. Populations of *D. noxia* in Asia, North America, and South America also have shown the ability to overcome the resistance of different *Dn* genes, especially *Dn4* (Puterka et al. 1992, Basky 2003, Hawley et al. 2003, Smith et al. 2004), yet the exact nature of *Dn* gene–*D. noxia* biotype interaction has not been determined. Jyoti et al. (2005) compared the development of North American biotypes 1 and 2 and related plant damage, noting that virulence in biotype 2 to *Dn4* and *Dny* is expressed more rapidly at 24°C than at 20°C. Nevertheless, determining the categories of resistance in potentially resistant germplasm provides information about the extent of selective pressures that a resistant cultivar may place on a *D. noxia* population and the durability of that resistance. Therefore, it is important to identify and categorize cereal germplasm with resistance to *D. noxia* biotype 2.

Although the location of a resistance gene on a chromosome is useful for marker-assisted selection, it is equally important to understand the category of resistance present in a germplasm to develop sustainable resistant cultivars. Resistance categories of antibiosis, antixenosis, and tolerance are normally recognized as defining resistance (Painter 1951). Antixenosis resistance occurs when the plant acts as a poor host and is not favored by the arthropod as food, shelter, or an oviposition site. Nonpreference results in reduced colonization of a plant by arthropods, thus reducing the losses caused by the pest (Pedigo 1999; Smith 2005). Antibiosis resistance occurs when the plant adversely affects the growth and development of the pest arthropod. For example, arthropod metabolism can be negatively affected either by the presence of certain chemicals or the absence of sufficient nutrients (Pedigo 1999, Smith 2005). These factors result in the reduced proliferation of the arthropod on a host plant and a considerable reduction in arthropod-related damage.

Unlike antixenosis and antibiosis resistance that involve plant–arthropod interactions, tolerance resistance is the ability of a plant to withstand arthropod damage and to yield significantly higher dry mass than the susceptible plant yields under similar conditions of infestation (Pedigo 1999, Smith 2005). Therefore, tolerance helps to raise the level at which the plant economic injury occurs and delays or negates the need for costly chemical control methods. Tolerance also may exert less selection pressure on the arthropod population to form new biotypes. When these advantages are considered, tolerance resistance is an attractive choice for incorporation into cultivars for durable arthropod resistance.

Dong et al. (1997) identified wheat cereal introduction (CItr) 2401 as resistant to *D. noxia* biotype 1 and detected two genes, each inherited as a dominant trait, that control this resistance. In preliminary plant phenotype screening experiments in 2003, we observed that CItr2401 is also resistant to *D. noxia* biotype 2. More recently, Collins et al. (2005) also identified biotype 2 resistance in CItr2401 as well as in 39 other wheat germplasm accessions. In these experiments, the level of biotype 2 resistance in CItr2401 as well as several other accessions was equivalent to that in plants of the resistant control wheat breeding line 94M370, which contains *Dn7.* In addition, Castro et al. (2005) identified antixenosis resistance linked to quantitative trait loci from *Triticum dicoccoides* × *Ageilops tauschii* synthetic wheat lines to a *D. noxia* population in Argentina virulent to *Dn2* and *Dn4.* The objectives of the experiments in our study were to categorize the resistance in CItr2401 and Stanton to *D. noxia* biotypes 1 and 2 and to confirm the mode of inheritance of the resistance in CItr2401 to biotype 2.

Materials and Methods

Plant and Insect Culture. Seeds of the wheat germplasm CItr2401 were obtained from the USDA Small Grains Repository, Aberdeen, ID. Seeds of the susceptible wheat 'Jagger' and 'Karl' as well as the biotype 1-resistant Stanton were obtained from the Kansas Crop Improvement Association, Manhattan, KS.

Biotype 1 *D. noxia* collected from wheat fields near Hays, KS, and biotype 2 individuals collected from wheat fields near Biggs, CO (via the USDA–ARS Plant Science Research Laboratory at Stillwater, OK), were cultured in separate locations on susceptible Jagger plants at Kansas State University for use in all experiments. The identity of each biotype was verified independently in diagnostic plant differential greenhouse assays at Stillwater and Manhattan. Voucher specimen no. 176 (*D. noxia* biotype 2) is located in the Kansas Sate University Museum of Entomological and Prairie Arthropod Research.

Tolerance, antixenosis, and antibiosis resistance in CItr2401 and Stanton were determined according to modifications to the methods of Flinn et al. (2001). Plants were grown in the greenhouse (24:20°C day/night and a photoperiod of 14:10 [L:D] h). In all experiments, plants were grown in Pro-Mix'Bx' potting

mix (Premier ProMix, Lansing, MI) in 16.5-cm-diameter plastic pots. Unless otherwise mentioned, experiments were conducted in the greenhouse at the same environmental conditions for optimal plant growth.

Tolerance. Tolerance was measured by calculating the proportional plant dry weight loss (DWT) and tolerance index (TI) of CItr2401, Stanton, Jagger, and Karl, where DWT = [(WC − WT)/WC × 100], WC is dry weight of noninfested control plant, WT is dry weight of infested plant, and TI = DWT/number of aphids produced on infested plants (Dixon et al. 1990, Reese et al. 1994). The TI was determined to compensate for the confounding effect of differing numbers of *D. noxia* on infested plants. Genotypes with TI values significantly lower than those of the susceptible controls Jagger or Karl were considered tolerant.

Pregerminated seeds of each genotype were planted in pots and allowed to grow to the two-leaf stage, and, within each genotype, 12 plants were paired for height and growth. In each pair of plants, a treatment plant was infested with three *D. noxia* biotype 1 or 2 (experiments with CItr2401, Stanton, and Jagger) or five *D. noxia* biotype 2 (experiments with CItr2401 and Karl), and the paired plant served as a noninfested control. Plants in experiments with CItr2401, Stanton, and Jagger were arranged in a randomized complete block design with each block containing one pair of plants of each genotype. Plants in experiments with CItr2401 and Karl were arranged in a completely randomized design.

In both sets of experiments, each pot was covered with a nylon mesh cage, and aphids were allowed to feed until the infested Jagger or Karl plants showed complete leaf rolling and 95% chlorosis of the youngest true leaf. Cages were then removed and aphids on each of the infested plants were collected on a sheet of wax paper, placed in 70% alcohol, and counted. The shoots from noninfested and infested plants were cut at the soil surface and placed in preweighed aluminum foil pouches. Roots were washed thoroughly to remove attached soil particles and were placed in similar preweighed aluminum foil pouches. The pouches with the shoots and roots were dried in an oven at 75°C for 3 and 7 d, respectively. Tissue weights were determined by subtracting the weight of the foil pouch from the combined pouch and tissue weight.

In the DWT experiments, we observed that infested CItr2401 plants showed very little or no leaf rolling in response to biotype 2, compared with susceptible Karl plants. To determine whether this trait is related to tolerance, CItr2401 plants were infested with two times the number of biotype 2 as on Karl plants, because we had observed that approximately twice the number of aphids were produced on Karl plants as on CItr2401 plants in the tolerance experiments. To determine whether CItr2401 plants had tolerance to leaf rolling and chlorosis, 11 CItr2401 plants and 11 Karl plants, each at the two-leaf stage of development, were infested with eight aphids (Karl) or 16 aphids (CItr2401), covered with a nylon mesh cage, and arranged in a completely randomized design. Two weeks after infestation, the plants were rated for leaf rolling and chlorosis as per the damage rating described by Smith et al. (1991).

Antixenosis. To determine antixenosis, seeds of each genotype were pregerminated in a petri dish, and one seedling of each was planted equidistantly at the periphery of a single pot. At the two-leaf stage of seedling growth, 10 pots (replicates) containing seedlings of each genotype were arranged in a completely randomized design and infested by releasing *D. noxia* into the center of each pot on a piece of Jagger wheat leaf. In experiments involving CItr2401, Stanton, and Jagger, each plant within a pot was infested at the rate of two (biotype 1) or one (biotype 2) aphids per plant. Plants were covered with nylon mesh cages, and the numbers of aphids on each plant were counted at 6, 12, and 24 h after infestation. In experiments with CItr2401 and Karl, one plant within a pot was infested with two biotype 2 aphids per plant and the number of aphids present on each plant at 1, 6, 12, 24 and 48 h after infestation was counted and recorded. The number of aphids on each genotype served to indicate the degree of antixenosis present.

McCloud and Berenbaum (1994) and Mazza et al. (1999) found that certain plants show increased resistance (reduced herbivory) in the presence of direct light and considerably less resistance when placed under greenhouse conditions that filter out UV radiation. Hence, we duplicated the biotype 2 antixenosis experiment involving CItr2401 and Karl outdoors to exclude potential greenhouse effects. This experiment was conducted under natural solar illumination, except that seedlings were grown in the greenhouse for 10 d, covered with a nylon mesh cage, and placed outdoors in a completely randomized design with 15 pots (replicates). At the two-leaf stage, cages were removed, seedlings were infested by placing 16 adult aphids in the center of the pot with a camel's-hair brush, and the cages were replaced. The rate of aphid infestation was greater than in the greenhouse experiment because the expected survival of aphids outdoors was not known. After 24 h, the cages were carefully removed, and the number of aphids on each plant was counted and recorded for further analysis.

Antibiosis. Antibiosis to biotypes 1 and 2 in CItr2401 and Stanton was determined by counting the numbers of *D. noxia* on the infested plants in each of the 12 pairs of plants in the DWT tolerance experiments involving CItr2401, Stanton, and Jagger. Antibiosis in CItr2401 was further assessed by calculating the intrinsic rate of increase (r_m) of single aphids on individual seedlings of CItr2401 and Karl, where $r_m = 0.738\ (\log e\ M_d)/d$ (d is time required for a newly emerged aphid [F_1] to produce its first offspring, M_d is total number of progeny produced by the mother of F_1 [P_1], and 0.738 is mean regression slope of the M_d over d for four aphid species; Wyatt and White 1977).

Seedlings of CItr2401 and Karl were planted in separate pots and the pots were arranged in completely randomized design with 10 replicates. When the plants reached the two-leaf stage, the midsection of one leaf was enclosed inside a cage made of an ≈2.5-cm-long section of plastic drinking straw. Cages had

Table 1. Mean ± SE percentage of proportional dry weight loss and mean tolerance index values for the roots of CItr2401, Stanton and Jagger wheat plants infested with *D. noxia* biotype 1 for 23 d

Genotype	Mean ± SE % DWT[a]		Mean ± SE TI[b]	
	Biotype 1	Biotype 2	Biotype 1	Biotype 2
Jagger	19.56 ± 10.25a	14.82 ± 12.58a	0.36 ± 0.19a	0.14 ± 0.05a
Stanton	−0.10 ± 0.05ab	20.75 ± 7.37a	−0.26 ± 0.14ab	0.10 ± 0.08a
CItr2401	−15.63 ± 7.71b	1.10 ± 7.42a	−0.82 ± 0.41b	0.06 ± 0.37a

Means followed by the same letter are not significantly different ($P > 0.05$, Fisher least square means).
[a] DWT = [(dry weight of control plant − dry weight of infested plant)/dry weight of control plant] × 100.
[b] TI = DWT/no. of aphids produced on infested plants.

been previously ventilated with 20–30 holes made by piercing the straw with an insect pin. The leaf tip was placed in one end of the straw cage, and the cage was moved toward the plant stem until the middle leaf section was enclosed. Each plant leaf was then infested with one late instar *D. noxia* biotype 2 nymph (P_1), and the ends of the cage were plugged with pieces of sponge.

The P_1 aphids were observed every 6 h during the day and every 12 h during the night to determine the production of nymphs. When P_1 produced its first nymph (F_1), the time was recorded. P_1 was then moved to a new leaf on the same plant and caged in a different drinking straw cage. When F_1 produced its first offspring, the time (d) was recorded. The number of nymphs produced by P_1 was counted and recorded. M_d and d were calculated for each of the aphids on CItr2401 and Karl plants, and the mean r_m of aphids on each plant genotype was calculated for analysis.

Inheritance of Resistance. To determine the inheritance of resistance in CItr2401, an F_2 population was created using Karl and CItr2401 as the female and male parents, respectively. The F_2 population was used to create an $F_{2:3}$ population and an F_4 population by using the single seed descent method. Two hundred seeds of the F_2 population, 250 families of the $F_{2:3}$ population (16 seeds per family), and 150 seeds of the F_4 population were planted in 53.5- by 35.5-cm plastic flats filled with Pro-Mix'Bx' potting mix and grown in the greenhouse at the conditions described previously. F_2 and F_4 seeds were planted individually, whereas $F_{2:3}$ population families were planted in rows in the flats. CItr2401 and Karl also were planted in each flat, as resistant and susceptible controls, respectively. At the two-leaf stage, plants were infested with *D. noxia* biotype 2 by placing a 0.5-cm-long piece of Jagger leaf containing four aphids at the base of each plant. Three weeks after infestation, when the susceptible control plants were dead or dying, plants were rated for damage as described by Smith et al. (1991).

Statistical Analyses. Data from category experiments were analyzed using a one-way analysis of variance (ANOVA) and PROC GLM (SAS Institute 1985). Means, when significant, were separated using Fisher least square means procedure ($P < 0.05$) for biotype 1 and 2 experiments involving CItr2401, Stanton, and Jagger; or the least significant difference (LSD) procedure ($P < 0.05$) for biotype 2 experiments involving CItr2401 and Karl. Inheritance data were subjected to chi-square analysis to determine the goodness-of-fit of the segregation ratios of different populations to the expected Mendelian segregation ratios.

Results

Tolerance. Shoot tissues from plants of Stanton, CItr2401, and Jagger were not significantly different from one another in tolerance to biotype 1, based on proportional DWT ($F = 1.00$; df = 2, 35; $P = 0.038$) or TI measurements ($F = 1.07$; df = 2, 35; $P = 0.356$) (data not shown). The DWT for shoots of CItr2401 plants infested with biotype 2 (−2.97) was significantly less ($P = 0.001$) than that of Jagger plant shoots (33.64) and was significantly less ($P = 0.006$) than that of Stanton plant shoots (26.11) (data not shown). Nevertheless, after correcting for the effect of differences in aphid populations between infested plants of the different genotypes, the TI values also demonstrated that there were no significant differences ($P = 0.803$) between the TI of shoots of Jagger (0.28), Stanton (0.17), and CItr2401 (0.51) infested with biotype 2.

Tolerance measurements involving plant roots provided different results. The mean DWT of roots from CItr2401 plants infested with biotype 1 was significantly lower than the DWT of roots of Jagger plants ($F = 5.68$; df = 2, 35; $P = 0.008$) (Table 1), although there were no significant differences between the root DWT of CItr2401 and Stanton, and the root DWT of Stanton and Jagger plants. The same trends held true for root TI measurements from plants infested with biotype 1. The TI (plant dry weight loss per aphid) for roots of CItr2401 plants was significantly reduced ($F = 4.81$; df = 2, 35; $P = 0.015$) compared with roots of Jagger plants (Table 1). There were no significant differences in TI root comparisons between CItr2401 and Stanton plants or between Stanton and Jagger plants.

In contrast, comparisons of CItr2401 and Karl infested with biotype 2 yielded no significant differences between the DWT of shoots of CItr2401 compared with that of Karl ($F = 0.63$; df = 1, 16; $P = 0.438$) (Fig. 1). However, the root DWT values were significantly less on CItr2401 plants infested with biotype 2 and than on Karl plants ($F = 12.97$; df = 1, 16; $P = 0.003$) (Fig. 1). This difference also was reflected in the whole plant (combined root and shoot) DWT of Karl (76.9), which was significantly greater than that

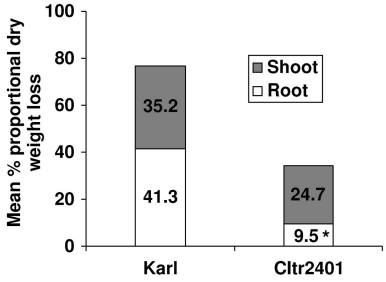

Fig. 1. Mean proportional DWT of roots and shoots of Karl and CItr2401 wheat plants infested with *D. noxia* biotype 2. Star indicates significant difference at $P > 0.05$; $n = 9$ (Karl), $n = 8$ (CItr2401).

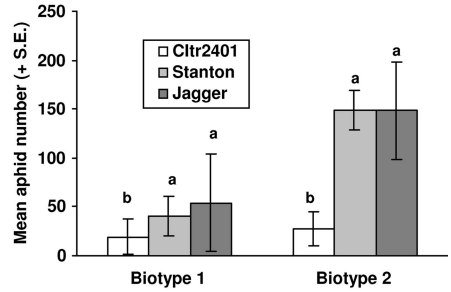

Fig. 3. Mean ± SE number of *D. noxia* biotype 1 and 2 present on Karl and CItr2401 wheat plants at 23 d (biotype 1) or 15 d (biotype 2) after infestation. Within each biotype, means followed by the same letter are not significantly different ($P > 0.05$, Fisher least square means).

of CItr2401 (34.2) ($F = 5.81$; df = 1, 16; $P = 0.029$) (Fig. 1).

In the experiments involving measurements of tolerance to leaf rolling, all Karl plants exhibited a completely rolled central leaf and partially rolled and chlorotic adjacent older leaves. In contrast, the central leaves of CItr2401 plants were partially rolled and the adjacent leaves had little or no rolling and only partial chlorosis, even under two-fold greater infestation (Fig. 2). These results demonstrate that although CItr2401 does not display tolerance when reduced proportional root or shoot dry weight losses are adjusted for aphid population differences, it does exhibit significant tolerance to leaf rolling and chlorosis, compared with Karl ($F = 73.33$; df = 1, 21; $P < 0.0001$ for leaf rolling; $F = 39.2$; df = 1, 21; $P < 0.0001$ for chlorosis).

Antixenosis. There were no differences in the preference of biotype 1 among CItr2401, Stanton, or Jagger at 6 h after infestation; however, CItr2401 and Stanton exhibited antixenosis at 12 h after infestation. The number of aphids on Jagger plants was significantly greater (df = 2, 29; $F = 6.20$; $P = 0.0063$) than that on CItr2401 or Stanton. By 24 h after infestation, however, these differences were nonsignificant (data not shown). Biotype 2 exhibited no preference between any of the three genotypes at 6, 12, or 24 h after infestation (data not shown).

Plants of CItr2401 also exhibited no antixenotic effects on *D. noxia* biotype 2 when compared with Karl in the greenhouse experiment at 1, 6, 12, 24, and 48 h after infestation (data not shown). Similar results were noted in the outdoor experiment. The mean number of biotype 2 on CItr2401 (6.33) and Karl plants (5.20) was not significantly different at 24 h postinfestation (data not shown). Therefore, we conclude that in both the greenhouse and in natural light, CItr2401 exhibits no antixenotic effects toward *D. noxia* biotype 2.

Antibiosis. At 23 d postinfestation, there were significantly fewer biotype 1 aphids on CItr2401 than on Stanton or Jagger plants ($F = 8.29$; df = 2, 35; $P = 0.0012$). However, there were no differences in the number of aphids between Stanton and Jagger plants (Fig. 3). The same pattern was evident at 15 d after infestation with biotype 2. CItr2401 plants contained significantly fewer aphids ($F = 31.63$, df = 2, 35; $P < 0.0001$) than did Jagger or Stanton plants (Fig. 3).

When biotype 2 *D. noxia* were confined on CItr2401 and Karl plants, there was no difference in the time required to reach reproductive maturity (Table 2). However, the number of progeny produced by biotype 2 on CItr2401 was significantly reduced in comparison with the number of progeny produced by biotype 2 on Karl ($F = 12.37$; df = 1, 19; $P = 0.003$) (Table 2). As a result, the r_m of aphids confined on the foliage of CItr2401 was significantly lower ($F = 6.63$, df = 1, 19; $P = 0.019$) than the r_m of aphids on the

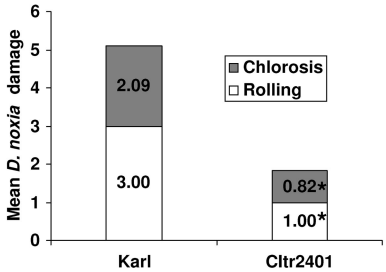

Fig. 2. Mean leaf rolling and chlorosis damage rating of Karl and CItr2401 wheat plants infested with *D. noxia* biotype 2. Star indicates significant difference at $P > 0.05$; $n = 11$.

Table 2. Mean ± SE prereproductive period, number of progeny produced by P_1 adults, and r_m of *D. noxia* biotype 2 on Karl and CItr2401 wheat plants

Genotype	Mean ± SE F_1 nymph prereproductive period (d)	Mean ± SE no. progeny produced by P_1 adults	Mean ± SE r_m [a]
Karl	11.4 ± 0.7a	28.3 ± 3.6a	0.11 ± 0.0570a
CItr2401	9.7 ± 0.9a	14.3 ± 0.01b	0.08 ± 0.006b

Means followed by the same letter are not significantly different ($P > 0.05$, LSD).

[a] $r_m = 0.738$ (log e M_d)/d, where d is time required for a newly emerged F_1 to produce first offspring, M_d is total progeny produced by the mother of F_1 (P_1), 0.738 is mean regression slope of M_d over d for four aphid species (Wyatt and White 1977).

Table 3. Segregation for resistance to *D. noxia* biotype 2 feeding damage in wheat plant populations derived from the cross Karl × CItr2401

Population	Phenotype	No. of plants		Expected ratio	χ^2 value	df	*P* value
		Expected	Observed				
F_2	Resistant	79.69	79	15	0.096	1	0.757*
	Susceptible	5.31	6	1			
F_4	Resistant	115.55	115	12.75	0.013	1	0.91*
	Susceptible	29.45	30	3.25			

* $P > 0.05$, fit to the expected segregation ratio for two dominant gene model.

foliage of Karl (Table 2). These results were validated by those obtained from tolerance experiments, in which the number of aphids on infested Karl plants (316.1) was significantly greater ($F = 14.39$, df = 1, 16; $P = 0.002$) than the mean number of aphids on infested CItr2401 plants (145.3). Therefore, we conclude that CItr2401 exerts significant antibiosis effects on *D. noxia* biotype 2, resulting in a reduced intrinsic rate of increase.

Inheritance of Resistance. Dong et al. (1997) determined that CItr2401 plants contain two genes that control resistance to *D. noxia* biotype 1 and that one of the genes is *Dn4.* However, Haley et al. (2004) found that biotype 2 is virulent to plants containing *Dn4.* We attempted to clarify the inheritance and number of biotype 2 resistance genes in CItr2401 by using a $F_{2:3}$ family population, but we were unable to do so, because 40% of the families did not germinate and those that did provided only one to 12 plants per family. Nevertheless, the F_2 segregation ratio for resistance to biotype 2 fit that of a 15:1 inheritance model for two dominant genes (Table 3). Similarly the F_4 segregation ratio for resistance fit the ratio expected for the inheritance of two dominant genes (Table 3). These inheritance results are in agreement with those of Dong et al. (1997) and indicate that two dominant genes control *D. noxia* biotype 2 resistance in CItr2401.

Discussion

Each of the three categories of resistance to *D. noxia* biotype 1 has been documented in different accessions of bread wheat, triticale (*Trticosecale* spp.), and barley (Souza 1998). Antixenosis was identified as an independent occurrence only in the accession PI225217 (Baker et al. 1994) and in synthetic hexaploid wheat accessions (Lage et al. 2004). Several accessions were identified with antixenosis, antibiosis, tolerance, or a combination of the three categories (Du Toit 1987, 1989; Formusoh et al. 1992; Smith et al. 1992). The results from our experiments fit the same trend, suggesting that biotype 2 will be subjected to similar selection pressures as biotype 1. Therefore, we propose that the sources of *D. noxia* resistance containing multiple categories should be employed in integrated pest management (IPM) strategies to delay the development of resistance-breaking biotypes.

The reduced leaf rolling and reduced chlorosis expressed by CItr2401 plants along with the two gene resistance of CItr2401 are all especially informative when compared with the phenotypic reactions of plants containing other *Dn* genes. Liu et al. (2005) found slight chlorotic streaking and no leaf rolling in plants containing *Dn1*, *Dn2*, *Dn5*, *Dn6*, *Dnx*, and in contrast found that plants containing *Dn4* exhibit sporadic chlorotic spots and slight leaf rolling. These two groups of phenotypic reactions correspond to *Dn* gene clusters on wheat chromosomes 7DS (*Dn1-Dnx*) and 1DS (*Dn4*) (Liu et al. 2005). The phenotypic reaction of CItr2401 plants includes both of these phenotypes. Therefore, the two resistance genes in CItr2401 plants could belong to one or both of the two gene clusters. The assertion of Dong et al. (1997) that one of the genes in CItr2401 is *Dn4* seems to be invalid, because Haley et al. (2004) demonstrated that *Dn4* is susceptible to biotype 2. The results of Dong et al. (1997) also may have been affected by a lack of seed purity in the CItr2401 sample used.

The differences in root and shoot tolerance resistance in CItr2401 to *D. noxia* suggest interesting scenarios in the development of evolutionary relationships between cereal plants and *D. noxia.* In response to *D. noxia* herbivory, it would have been advantageous for plants to translocate fresh weight from shoot to root systems, leading to the death of shoots and causing aphids to starve and eventually die. Plants could subsequently produce new shoots, using carbohydrates stored in the root system. This type of damage escape may have been especially useful when dealing with arthropods such as aphids that have a relatively short life cycle. Therefore, we suggest that the measurement of root and shoot dry weights are a more accurate measure of plant tolerance to *D. noxia* infestation than whole plant dry weights alone.

From a practical standpoint, the results of the DWT and TI tolerance experiments demonstrate the significance of, and need for, separate root and shoot DWT measurements of tolerance to *D. noxia* infestation. Zwer et al. (1994) demonstrated that wheat root development is significantly affected by *D. noxia* infestation and concluded that root measurements in conjunction with measurements of leaf damage symptoms were necessary to identify promising *D. noxia* resistant genotypes.

The fact that two genes seem to be inherited as dominant traits in control of resistance to *D. noxia* biotype 2 in CItr2401 is of interest, and suggests the possibility that separate genes control the expression of antibiosis and tolerance. The expression of antibi-

osis resistance in CItr2401 was exhibited as a significantly reduced intrinsic rate of increase, when based on reproduction by one female. These results were paralleled by the results of aphid population increase experiments in which CItr2401 plants infested with a group of five biotype 2 yielded significantly fewer aphids than did Karl plants. This confirmation provides additional data for further analysis of the hypothesis of Qureshi and Michaud (2005) that *D. noxia* may better use host nutrients when feeding in groups than when feeding as individuals.

When antibiosis effects were factored out, the combined TI measurement for shoots and roots of Karl and CItr2401 plants infested with biotype 2 did not differ significantly ($F = 0.01$; df = 1, 16; $P = 0.924$) (data not shown). Therefore, we conclude that the significantly reduced root DWT in CItr2401 in response to biotype 1 feeding is linked to tolerance, because TI values for CItr2401 were significantly less than those on Jagger. Conversely, the lack of differences for either root DWT or root TI between CItr2401, Stanton, or Jagger plants infested with biotype 2 (Table 1) indicates that resistance to biotype 2 in CItr2401 is due to a greatly decreased r_m of biotype 2.

No significant antixenotic effects were detected in CItr2401 at 24 or 48 h postinfestation. Although tolerance was not evident in proportional dry weight loss measurements of roots or shoots adjusted for aphid population size, CItr2401 plants exhibited reduced leaf rolling, compared with susceptible Karl plants. The leaf rolling displayed by infested susceptible plants normally provides *D. noxia* a tubular refuge from insecticides and biological control agents (Zwer et al. 1994, Lage et al. 2004). Thus, tolerance to leaf rolling may be advantageous from an IPM perspective, because the reduced leaf rolling of CItr2401 plants is likely to decrease *D. noxia* biotype 2 infestations by allowing chemical and biological controls to function normally and may assist in maintaining normal photosynthesis and growth during *D. noxia* infestation.

Acknowledgments

This research was supported by a U.S. Egypt Science and Technology Board Grant BI07-001-018, by USDA–ARS Areawide Wheat Pest Management Grant AR-2472, and by the Kansas Crop Improvement Association. Contribution 06-209-J of the Kansas Agricultural Experiment Station.

References Cited

Assad, M. T. 2002. Inheritance of resistance to the Russian wheat aphid in an Iranian durum wheat line. Plant Breed. 121: 180–181.

Baker, C. A., M. R. Porter, and J. A. Webster. 1994. Inheritance and categories of Russian wheat aphid (Homoptera: Aphididae) resistance in STARS-225217, a red winter wheat, pp. 148–157. *In* S. S. Quisenberry and F. B. Peairs [eds.], A response model for an introduced pest—the Russian wheat aphid. Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD.

Basky, Z. 2003. Biotypic and pest status differences between Hungarian and South African populations of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae). Pest Manag. Sci. 59: 1152–1158.

Castro, A. M., A. Vasicek, M. Manifiesto, D. O. Giménez, M. S. Tacaliti, O. Dobrovolskaya, M. S. Röder, J. W. Snape, and A. Börner. 2005. Mapping antixenosis genes on chromosome 6A of wheat to greenbug and to a new biotype of Russian wheat aphid. Plant Breed. 124: 229–233.

Collins, M. B., S. D. Haley, F. B. Peairs, and J. B. Rudolph. 2005. Biotype 2 Russian wheat aphid resistance among wheat germplasm accessions. Crop Sci. 45: 1877–1880.

Dixon, A.G.O., P. J. Brammel-Cox, J. C. Reese, and T. L. Harvey. 1990. Mechanism of resistance and their interactions in twelve sources of resistance biotype E greenbug (Homoptera: Aphididae). J. Econ. Entomol. 83: 2324–240.

Dong, H., J. S. Quick, and Y. Zhang. 1997. Inheritance and allelism of Russian wheat aphid resistance in several wheat lines. Plant Breed. 116: 449–453.

Du Toit, F. 1987. Resisatnce in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Homoptera: Aphididae). Cereal Res. Commun. 15: 175–179.

Du Toit, F. 1989. Components of resistance in three bread wheat lines to Russian wheat aphid (Homoptera: Aphididae) in South Africa. J. Econ. Entomol. 82: 1779–1781.

Estakhr, A., and M. T. Assad. 2002. The allelic relationships among Russian wheat aphid genes in Iranian wheat lines and known genes. J. Agric. Sci. 138: 281–284.

Flinn, M., C. M. Smith, J. C. Reese, and B. S. Gill. 2001. Categories of resistance to greenbug (Homoptera: Aphididae) biotype I in *Aegilops tauschii* germplasm. J. Econ. Entomol. 94: 558–563.

Formusoh, E. S., G. E. Wilde, J. H. Hatchett, and R. D. Collins. 1992. Resistance to Russian wheat aphid (Homoptera: Aphididae) in Tunisian wheats. J. Econ. Entomol. 85: 2505–2509.

Fouche, A., R. L. Verhoeven, P. H. Hewitt, M. C. Walters, C. F. Kriel, and J. De Jager. 1984. Russian aphid (*Diuraphis noxia*) feeding damage on wheat, related cereals and a *Bromus* grass species, pp. 22–33. *In* M. C. Walters [ed.], Progress in Russian wheat aphid (*Diuraphis noxia* Mordw.) research in the Republic of South Africa. Department of Agriculture Technical Communication 191, Republic of South Africa.

Girma, M., G. Wilde, and J. C. Reese. 1990. Influence of temperature and plant growth stage on development, reproduction, life span, and intrinsic rate of increase of the Russian wheat aphid (Homoptera: Aphididae). Environ. Entomol. 19: 1438–1442.

Haley, S. D., F. B. Peairs, C. B. Walker, J. B. Rudolph, and T. L. Randolph. 2004. Occurrence of a new Russian wheat aphid biotype in Colorado. Crop Sci. 44: 1589–1592.

Hawley, C. J., F. B. Peairs, and T. L. Randolph. 2003. Categories of resistance at different growth stages in Halt, a winter wheat resistant to Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 96: 214–219.

Hein, G. L. 1992. Influence of plant growth on Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae). Reproduction and damage symptom expression. J. Kans. Entomol. Soc. 65: 369–376.

Hewitt, P. H., G.J.J. van Niekerk, M. C. Walters, C. F. Kriel, and A. Fouche. 1984. Aspects of the ecology of the Russian wheat aphid, *Diuraphis noxia*, in the Bloemfontein district. I. The colonization and infestation of sown wheat, identification of summer hosts and cause of infestation systems. pp. 3–13. *In* M. C. Walters [ed.], Progress in Russian wheat aphid (*Diuraphis noxia* Mordw.) research in the Re-

public of South Africa. Department of Agriculture Technical Communication 191, Republic of South Africa.

Johnson, V. A., L. W. Briggle, J. D. Axtell, L. F. Bouman, E. R. Leng, and T. H. Johnson. 1978. Grain crops, pp. 1–28. *In* M. Milner [ed.], Protein resources and technology. AVI Publ. Co., Westport, CT.

Jyoti, J. L., J. A. Qureshi, J. P. Michaud, and T. J. Martin. 2005. Virulence of two Russian wheat aphid biotypes to eight wheat cultivars at two temperatures. Crop Sci. 46: 774–780.

Lage, J., B. Skovmand, and S. B. Andersen. 2004. Resistance categories of synthetic hexaploid wheats resistant to the Russian wheat aphid (*Diuraphis noxia*). Euphytica 136: 291–296.

Liu, X. M., C. M. Smith, and B. S. Gill. 2002. Identification of microsatellite markers linked to Russian wheat aphid resistance genes Dn4 and Dn6. Theor. Appl. Genet. 104: 1042–1048.

Liu, X. M., C. M. Smith, B. R. Friebe, and B. S. Gill. 2005. Molecular mapping and allelic relationships of Russian wheat aphid-resistance genes. Crop Sci. 45: 2273–2280.

Martin, T. J., A. Fritz, and J. P. Shroyer. 2001. Stanton hard red winter wheat. Kansas State University Agricultural Experiment Station and Cooperative Extension Service Publication L-921.

Mazza, C. A., J. Zavala, A. L. Scopel, and C. L. Ballare. 1999. Perception of solar UVB radiation by phytophagous insects: behavioral responses and ecosystem implications. Proc. U.S. Natl. Acad. Sci. 96: 980–985.

McCloud, E. S., and M. R. Berenbaum. 1994. Stratospheric ozone depletion and plant-insect interactions: effects of UVB radiation on foliage quality of *Citrus jambhiri* for *Trichoplusia ni.* J. Chem. Ecol. 20: 525–539.

Morrison, W. P., and F. B. Peairs. 1998. Response model concept and economic impact, pp. 1–11. *In* S. S. Quisenberry and F. B. Peairs [eds.], A response model for an introduced pest—the Russian wheat aphid. Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD.

Painter, R. H. 1951. Insect resistance in crop plants. MacMillan, New York.

Pedigo, L. P. 1999. Entomology and pest management, 3rd ed. Prentice-Hall, Saddle River, NJ.

Pike, K. S., D. Allison, L. K. Tanigoshi, R. F. Harwood, S. L. Clement, S. E. Halbert, C. M. Smith, J. B. Johnson, G. L. Reed, and P. K. Zwer. 1991. Russian wheat aphid-biology, damage and management. Pacific Northwest Ext. Publ. PNW371.

Prinsloo, G. J. 2000. Host and host instar preference of *Aphelinus* sp. nr. *varipes* (Hymenoptera: Aphelinidae), a parasitoid of cereal aphids (Homoptera: Aphididae) in South Africa. Afr. Entomol. 8: 57–61.

Puterka, G. J., J. D. Burd, and R. L. Burton. 1992. Biotypic variation in a worldwide collection of Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85: 1497–1506.

Qureshi, J. A., and J. P. Michaud. 2005. Comparative biology of three cereal aphids on TAM 107 wheat. Environ. Entomol. 34: 27–36.

Quick, J. S., G. E. Ellis, R. M. Norman, J. A. Stromberger, J. F. Shanahan, F. B. Peairs, J. B. Randolph, and K. Lorenz. 1996. Registration of 'Halt' wheat. Crop Sci. 36: 210.

Reese, J. C., J. R. Schwenke, P. S. Lamont, and D. D. Zehr. 1994. Importance and quantification of plant tolerance in crop pest management programs for aphids: greenbug resistance in Sorghum. J. Agric. Entomol. 11: 255–270.

SAS Institute. 1985. SAS user's guide: basics. SAS Institute. Cary, NC.

Smith, C. M. 2005. Plant resistance to arthropods—molecular and conventional approaches. Springer, Berlin, Germany.

Smith, C. M., D. Schotzko, R. S. Zemetra, E. J. Souza, and S. Schroeder-Teeter. 1991. Identification of Russian wheat aphid (Homoptera: Aphididae) resistance in wheat. J. Econ. Entomol. 84: 328–332.

Smith, C. M., D. Schotzko, R. S. Zemetra, and E. J. Souza. 1992. Categories of resistance to the Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85: 1480–1484.

Smith, C. M., T. Belay, C. Stauffer, P. Stary, I. Kubeckova, and S. Starkey. 2004. Identification of Russian wheat aphid (Homoptera: Aphididae) biotypes virulent to the Dn4 resistance gene. J. Econ. Entomol. 97: 1112–1117.

Souza, E. J. 1998. Host plant resistance to Russian wheat aphid (Homoptera: Aphididae) in wheat and barley, pp. 122–147. *In* S. S. Quisenberry and F. B. Peairs [eds.], A response model for an introduced pest—the Russian wheat aphid. Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD.

Souza, E., N. A. Bosque-Perez, D. J. Schotzko, M. J. Guttieri, and K. O'Brien. 2002. Registration of three wheat germplasms resistant to *Diuraphis noxia.* Crop Sci. 42: 319a-320.

Stoetzel, M. B. 1987. Information on and identification of *Diuraphis noxia* (Homoptera: Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. J. Econ. Entomol. 80: 696–704.

Walters, M. C., F. Penn, F. duToit, T. C. Botha, K. Aalbersberg, P. H. Hewitt, and S. W. Broodryk. 1980. The Russian wheat aphid, farming in South Africa, Leafl. Series, Wheat C3, Government Printer, Pretoria, 6.

Webster, J. A., and P. Kenkel. 1999. Benefits of managing small-grain pests with plant resistance, pp. 87–114. *In* B. R. Wiseman and J. A. Webster [eds.], Economic, environmental and social benefits of resistance in field crops. Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD.

Webster, J. A., K. J. Starks, and R. L. Burton. 1987. Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. J. Econ. Entomol. 80: 944–949.

Wraight, S. P., T. J. Poprawski, W. L. Meyer, and F. B. Peairs. 1993. Natural enemies of Russian wheat aphid (Homoptera: Aphididae) and associated cereal aphid species in spring-planted wheat and barley in Colorado. Environ. Entomol. 22: 1383–1391.

Wyatt, I. J., and P. F. White. 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. Appl. Ecol. 14: 757–766.

Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14: 415–421.

Zwer, P. K., M. G. Mosaad, A. A. Elsidaig, and R. W. Rickman. 1994. Effect of Russian wheat aphid on wheat root and shoot development in resistant and susceptible genotypes. Crop Sci. 34: 650–655.

Received 13 March 2006; accepted 7 June 2006.